

We claim:

1. An isolated and/or recombinant cell- or tissue-specific F-box protein.
- 5 2. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, which is expressed specifically in muscle tissue.
- 10 3. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 2, which protein comprises an amino acid sequence identical or homologous to the amino acid sequence shown in Figure 5B.
- 15 4. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, wherein expression of the F-box protein is at least 2-fold higher in one cell- or tissue-type as compared to another cell- or tissue-type.
- 20 5. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 2, which protein comprises an amino acid sequence encoded by a nucleic acid which hybridizes under stringent conditions to the nucleotide sequence set forth in Figure 5A.
- 25 6. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, which protein has an endogenous ubiquitin ligase activity and/or is a component of a ubiquitin ligase.
7. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 6, wherein the polypeptide mediates ubiquitination of a cell-type specific or tissue-type specific substrate protein.
- 30 8. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, which polypeptide is capable of interacting with at least one other protein selected from the group consisting of ubiquitin, a component of ubiquitin ligase, a skp1 protein, a cullins protein, an Rbx1 protein and a ubiquitin conjugating enzyme.

9. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, which polypeptide is at least 75% homologous to the amino acid sequence shown in Figure 5B.

5 10. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, which polypeptide is a vertebrate F-box protein.

11. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 10, which polypeptide is a mammalian F-box protein.

10 12. The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 11, which polypeptide comprises the amino acid sequence set forth in Figure 5B.

15 13. An isolated nucleic acid comprising a nucleotide sequence encoding a cell- or tissue-specific F-box polypeptide, or a nucleotide sequence complementary thereto, said F-box polypeptide including an amino acid sequence identical or homologous to the amino acid sequence set forth in Figure 5B, or a portion thereof.

20 14. The nucleic acid of claim 13, which nucleic acid hybridizes under stringent conditions to a nucleic acid probe comprising a nucleotide sequence represented by at least 60 consecutive nucleotides of the sequence shown in Figure 5A, or a sequence complementary thereto.

25 15. The nucleic acid of claim 14, which nucleic acid comprises the nucleotide sequence set forth in Figure 5A.

16. An isolated nucleic acid comprising a nucleotide sequence encoding a vertebrate cell- or tissue-specific F-box polypeptide.

30 17. The nucleic acid of claim 13 or 16, further comprising a transcriptional regulatory sequence operably linked to said nucleotide sequence so as to render said nucleic acid suitable for use as an expression vector.

35 18. An expression vector, capable of replicating in at least one of a prokaryotic cell and eukaryotic cell, comprising the expression vector of claim 17.

19. A host cell transfected with the expression vector of claim 18 and expressing said recombinant polypeptide.

5 20. A method of producing a recombinant cell- or tissue-specific F-box polypeptide comprising culturing the cell of claim 19 in a cell culture medium to express said recombinant polypeptide and isolating said recombinant polypeptide from said cell culture.

10 21. A transgenic animal having cells which harbor a transgene comprising the nucleic acid of claim 13 or 16, or in which a gene comprising said nucleic acid is disrupted.

15 22. An isolated nucleic acid which selectively hybridizes under high stringency conditions to at least ten nucleotides of the sequence set forth in Figure 5A or complementary sequences thereof, which nucleic acid can specifically detect or amplify a nucleic acid sequence of a vertebrate cell- or tissue-specific F-box gene.

20 23. The nucleic acid of claim 22, which is nucleic acid is labeled.

25 24. A reconstituted protein mixture comprising a cell- or tissue-specific F-box polypeptide and a substrate protein.

30 25. The reconstituted protein mixture of claim 24, wherein the cell- or tissue-specific F-box polypeptide is atrophin-1.

25 26. The reconstituted protein mixture of claim 24, wherein the substrate protein is a regulatory component of a muscle cell or a component of the myofibrillar apparatus.

30 27. An assay for identifying an inhibitor of cell- or tissue-specific F-box protein-mediated ubiquitination, comprising:

35 (i) providing a ubiquitin-conjugating system including a substrate polypeptide, an SCF complex including one or more cell- or tissue-specific F-box polypeptides and ubiquitin, under conditions which promote ubiquitination of the substrate polypeptide by the SCF complex;

 (ii) contacting the ubiquitin-conjugating system with a candidate agent;

(iii) measuring a level of ubiquitination of the substrate polypeptide in the presence of the candidate agent; and
(iv) comparing the measured level of ubiquitination in the presence of the candidate agent with ubiquitination of the substrate polypeptide in the absence of the candidate agent,
5 wherein a statistically significant decrease in ubiquitination of the substrate polypeptide in the presence of the candidate agent is indicative of an inhibitor of cell- or tissue-specific F-box protein-mediated ubiquitination.

10 28. The assay of claim 27, wherein the ubiquitin-conjugating system comprises a reconstituted protein mixture.

29. The assay of claim 27, wherein the ubiquitin-conjugating system comprises a cell lysate.

15 30. The assay of claim 27, wherein the SCF complex includes a polypeptide comprising the sequence set forth in Figure 5B.

20 31. The assay of claim 27, wherein the substrate protein is a regulatory component of a muscle cell or a component of the myofibrillar apparatus.

32. The assay of claim 27, wherein the ubiquitin is provided in a form selected from a group consisting of:
25 (i) an unconjugated ubiquitin, in which case the ubiquitin-conjugating system further comprises an E1 ubiquitin-activating enzyme (E1), an E2 ubiquitin-conjugating enzyme (E2), and adenosine triphosphate;
(ii) an activated E1:ubiquitin complex, in which case the ubiquitin-conjugating system further comprises an E2;
(iii) an activated E2:ubiquitin complex; and
30 (iv) an activated E3:ubiquitin complex.

33. The assay of claim 27, wherein the ubiquitin-conjugating system further comprises an E2 ubiquitin conjugating enzyme.

35 34. The assay of claim 27, wherein at least one of the ubiquitin and the substrate polypeptide comprises a detectable label, and the level of ubiquitination of the

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substrate polypeptide is quantified by detecting the label in at least one of the substrate polypeptide, the ubiquitin, and ubiquitin-conjugated substrate polypeptide.

35. The assay of claim 34, wherein the label group is selected from a group consisting of
5 radioisotopes, fluorescent compounds, enzymes, and enzyme co-factors.

36. The assay of claim 34, wherein the detectable label comprises a polypeptide having a measurable activity, and the substrate polypeptide is fusion protein including the detectable label.
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37. The assay of claim 27, wherein the amount of ubiquitination of the substrate polypeptide is quantified by an immunoassay.
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38. The assay of claim 27, wherein the amount of ubiquitination of the substrate polypeptide is quantified by chromatography or electrophoresis.
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39. The assay of claim 27, wherein the ubiquitin-conjugating system comprises a host cell expressing the substrate polypeptide and the SCF complex.
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40. The assay of claim 39, wherein the host cell expresses a recombinant cell- or tissue-specific F-box polypeptide.
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41. The assay of claim 27, wherein the ubiquitin-conjugating system further comprises a cullins polypeptide, a Skp1 polypeptide and/or an Rbx1 polypeptide.
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42. An assay for identifying an inhibitor of an interaction between a substrate polypeptide and an SCF complex including a cell- or tissue-specific F-box protein, comprising:
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- (i) providing a reaction system including the substrate polypeptide and an SCF complex including a cell- or tissue-specific F-box protein, wherein the substrate polypeptide and the SCF complex interact;
- (ii) contacting the reaction system with a candidate agent;
- (iii) measuring formation of complexes containing the substrate polypeptide and the SCF complex in the presence of the candidate agent; and

(iv) comparing the measured formation of complexes in the presence of the candidate agent with complexes formed in the absence of the candidate agent,

5 wherein a statistically significant decrease in the formation of complexes in the presence of the candidate agent is indicative of an inhibitor of the interaction of the substrate polypeptide and the SCF complex.

10 43. The assay of claim 42, wherein the cell- or tissue-specific F-box protein is atrohpin-1.

15 44. The assay of claim 42, wherein the substrate is a regulatory component of a muscle cell or a component of the myofibrillar apparatus.

45. The assay of claim 42, wherein the reaction system comprises a reconstituted protein mixture.

20 46. The assay of claim 42, wherein the reaction system comprises a cell lysate.

47. The assay of claim 42, wherein the reaction system comprises a cell.

25 48. The assay of claim 42, wherein the substrate polypeptide and the cell- or tissue-specific F-box protein are provided as fusion proteins in an interaction trap system.

49. The assay of claim 27, which comprises a further step of preparing a pharmaceutical preparation of one or more compounds identified as inhibitors of cell- or tissue-specific F-box protein-mediated ubiquitination.

25 50. A method for diagnosing a muscle wasting disorder in a patient, comprising:

30 (i) ascertaining the level of expression of an F-box polypeptide comprising the sequence set forth in Figure 5B in a sample of muscle cells from the patient; and
(ii) diagnosing the presence or absence of a muscle wasting disorder utilizing, at least in part, the ascertained level of expression or activity of the F-box polypeptide;

wherein an increased level of expression of the F-box polypeptide or F-box polypeptide-dependent ubiquitination activity in the sample, relative to a control sample of non-muscle cells, correlates with the presence of a muscle wasting disorder.

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10 51. The method of claim 50, wherein the patient is suffering from cachexia, cachexia secondary to infection or malignancy, cachexia secondary to human acquired immune deficiency syndrome AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, allograft rejections, Crohn's disease, ulcerative colitis, or pyresis, multiple sclerosis, autoimmune diabetes, systemic lupus erythematosus, prolonged inactivity or prolonged exposure to a microgravity environment.

15 52. The method of claim 50, further comprising the step of applying a treatment to the patient which inhibits the expression and/or activity of the F-box polypeptide.

20 53. The method of claim 52, wherein the muscle wasting disorder is associated with cachexia, cachexia secondary to infection or malignancy, cachexia secondary to human acquired immune deficiency syndrome AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, allograft rejections, Crohn's disease, ulcerative colitis, or pyresis, multiple sclerosis, autoimmune diabetes, systemic lupus erythematosus, prolonged inactivity or prolonged exposure to a microgravity environment.

25 54. A method for treating a patient suffering from a muscle wasting disorder, comprising administering to the patient an amount of an atrophin-1 inhibitor effective to inhibit the expression and/or activity of atrophin-1.

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55. The method of claim 54, wherein the muscle wasting disorder is associated with chachexia, cachexia secondary to infection or malignancy, cachexia secondary to human acquired immune deficiency syndrome AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, gouty arthritis, sepsis, septic shock, endotoxic shock, gram negative sepsis, toxic shock syndrome, adult respiratory distress syndrome, cerebral malaria, chronic pulmonary inflammatory disease, silicosis, pulmonary sarcoidosis, bone resorption diseases, reperfusion injury, graft vs. host reaction, allograft rejections, Crohn's disease, ulcerative colitis, or pyresis, multiple sclerosis, autoimmune diabetes, systemic lupus erythematosus, prolonged inactivity or prolonged exposure to a microgravity environment.

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54. A method for maintaining or increasing the muscle mass of an animal, comprising administering to the animal an amount of an atrophin-1 inhibitor effective to inhibit the expression and/or activity of atrophin-1.

55. The method of claim 54, wherein the animal is a human.

56. The method of claim 54, wherein the animal is a livestock animal.

57. The method of claim 56, wherein the animal is a cow, pig, goat or sheep.

58. A method for stimulating the proliferation of muscle stem cells, comprising contacting the stem cells with a compound capable of inhibiting the expression and/or activity of atrophin-1.

59. A transgenic livestock animal which is disrupted for atrophin-1.

60. A method for inhibiting protein degradation in muscle tissue of a patient without substantially affecting protein degradation in other tissues, comprising administering

to the patient an amount of an atrophin-1 inhibitor effective to inhibit the expression and/or activity of atrophin-1.